# Expression of cyclin A in intestinal biopsies from children with celiac disease

A. GRZANKA[1*], A. SZAFLARSKA-POPLAWSKA[2], A. ZURYN[1], D. GRZANKA[3]

*[1]Department of Histology and Embryology, Nicolaus Copernicus University, Collegium Medicum, Bydgoszcz, Poland, e-mail: agrzanka@cm.umk.pl, [2]Department and Clinic of Pediatrics, Allergology and Gastroenterology, Nicolaus Copernicus University, Collegium Medicum, Bydgoszcz, Poland; [3]Department of Clinical Pathomorphology, Nicolaus Copernicus University, Collegium Medicum, Bydgoszcz, Poland*

**Received 27 March, 2008**

The aim of this study was to determine the expression of cyclin A and describe its distribution in biopsy samples taken from children with suspected and confirmed celiac disease as well as in control samples. Investigated material consisted of 37 intestinal biopsies: 19 taken from patients with confirmed celiac disease, 9 from patients with its suspicion and 9 from healthy patients, who served as control. Immunohistochemical and immunogold methods were used to estimate cyclin A expression. In celiac disease samples morphological changes in epithelial cells, typical for disease, were shown. We observed weaker cyclin A expression, however there were also some cells with strong labeling in cytoplasm, near the nucleus. In control and suspected celiac disease groups cyclin A was present in the brush border, nucleus and whole cytoplasm, especially in proximity to the nucleus. In conclusion, these studies enabled us visualized pattern of distribution of cyclin A but let us also to presume that observed decrease of expression and its distribution might function as additional factor which could be taken under consideration to establish terminal diagnosis. We are aware of the fact that these are very first observations and that this subject needs to be further investigated with the use of additional methods and samples.

*Key words: celiac disease, cyclin A, immunogold, immunohistochemistry*

The gluten-sensitive enteropathy, also known as celiac disease, is a permanent intolerance to ingested gluten that results in immunologically mediated inflammatory damage to the small-intestinal mucosa [1].The mechanism of the celiac disease is still unclear, but some of its characteristic features as hypersensitivity to gluten resulting in destruction of the villus epithelial cells and crypt hyperplasia are known. Early diagnosis and management are important for the patients with this disease. In particular that patients with non-treated celiac disease have increased risk of malignancy, especially lymphomas. The most of previous studies have linked villous atrophy with enterophaty –type T-cell lymphoma (ETTL) [2–5]. Smedby et al showed for the first time that celiac disease is also associated with B-cell lymphomas [6]. Detection and right treatment of this disease with gluten – free diet may let patients avoid many severe complication. Because of the fact that clinical symptoms of the disease are not always cleary shown, diagnosis should be confirmed by additional methods. Serological tests for antibodies against endomysium, transglutaminase, and gliadin which have become the preferred way of diagnosing patients [7]. However, there is a constant effort to find a new diagnostic marker or technologies to improve diagnosis of this disease. Identification of new molecular markers associated with histological changes occurring in obtained biopsies from celiac disease may help pathologists in its estimation.

Cyclin A is a protein involved in cell cycle progression by activating the cyclin-dependent kinases (cdk2, or cdk1). The cyclin A/cdk2 complex activity is required for the regulation of S phase progression and complex of cyclin A/cdk1 for G2/M transition [8, 9]. It is well known that cyclin A plays a very important role during the cell cycle and some reports suggested that cyclinA/cdk2 complex controls DNA replication [10, 11]. There are also studies showing activation of the cyclinA/cdk2 during centrosome duplication or centri-

* Corresponding author

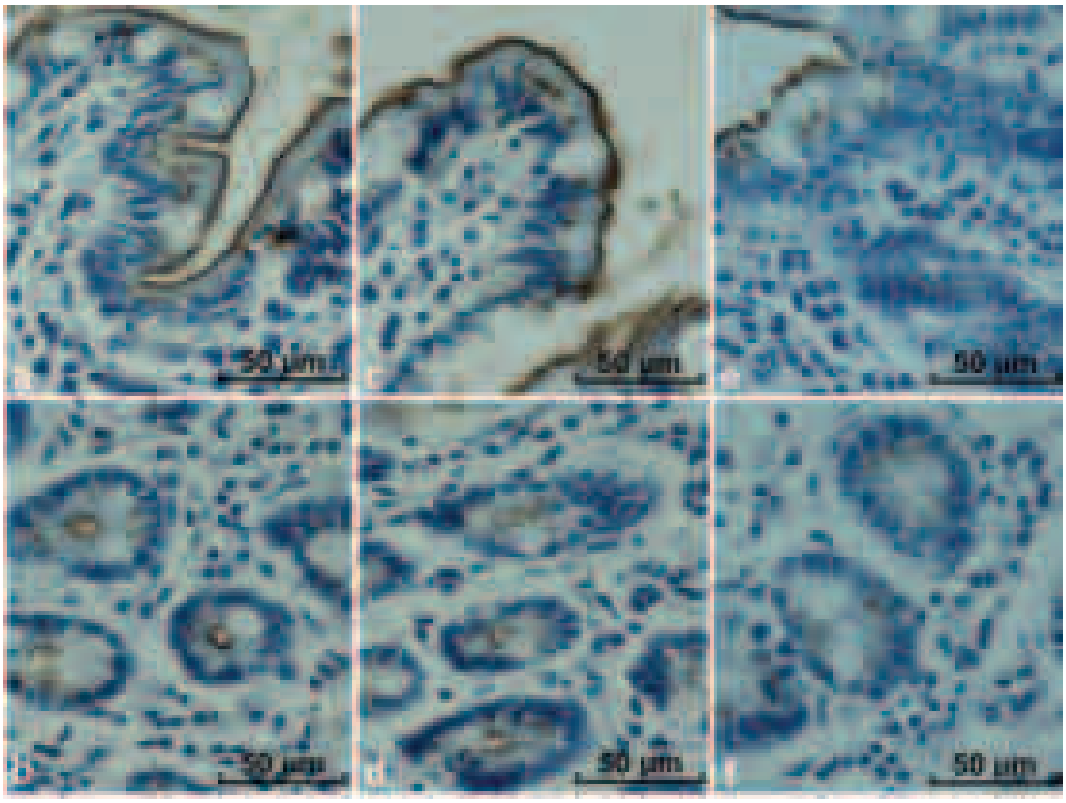


**Figure 1. Cyclin A expression in enterocytes in villi (A, C, E) and in undifferentiated columnar cells in crypts (B, D, F). Biopsy specimens of: control group (A,B), suspected (C,D) and confirmed celiac disease (E, F), stained with monoclonal anti-cyclin A antibody. (internal scale marker: 50 µm, DAB stain).**

ole separation [12–14]. Some authors have showed the relationship between the level of cyclin A or cdk expression and apoptosis and also a link between cyclin A subcellular localization and its cell functions [15–18]. Cyclin A is typically coexpressed with proliferation markers such as PCNA and Ki-67. Increased expression of cyclin A has been detected in many types of cancers (for example: leukaemia, lymphoma, liver cancer, lung cancer, ovarian, smooth muscle cancer) and has a poor prognosis [19–24]. Transgenic mice over expressing the wild type of nondegradable cyclin A in the mammary glands exhibit hyperplasia and nuclear abnormalities suggesting of preneoplastic alterations [20]. After conducting a review of the literature on cyclins, it seems to be worth to demonstrate our data concerning cyclin A. Our aim was to obtain information about its expression and distribution in studied samples.

## Material and methods

*Samples.* Investigated material consisted of 37 intestinal biopsies, taken from children hospitalized in the Department and Clinic of Pediatric, Allergology and Gastroenterology. 19 biopsies came from patients with confirmed celiac disease, 9 from patients with its suspicion and 9 normal biopsies were used as a control group. In case of patients with suspected celiac disease IgA endomysial test was positive but there were not present any histological changes, characteristic for celiac disease, in biopsies.

*Light microscopy.* For light microscopy, biopsy specimens were fixed in 4%PHA in PBS, dehydrated, embedded in paraffin and serially sectioned. Cyclin A was detected by the VECTASTAIN UNIVERSAL Elite ABC KIT. Sections were deparaffinized and hydrated through xylenes and graded alcohol series. Endogenous peroxidase activity was blocked with 3% $H_2O_2$. Sections were treated with monoclonal anti-cyclin A antibody produced in mouse (Sigma) diluted 1:50 in phosphate-buffered saline for 1 h. Then, they were incubated for 30 minutes with VECTASTAIN elite ABC Reagent. After that, the sections were developed with DAB Chromogen (Dako) for 5 minutes and counterstained with Meyer's hematoxylin. Controls specimens were incubated with noimmune antiserum.

*Electron microscopy.* For electron microscopy biopsies were cut into small fragments and fixed in 4% PHA in PBS for 1h at room temperature. Dehydration was performed in an ascending series of ethanol and than samples were embedded in LR White. For showing cyclin A postembedding streptavidin gold method was used. Sections were cut at 60

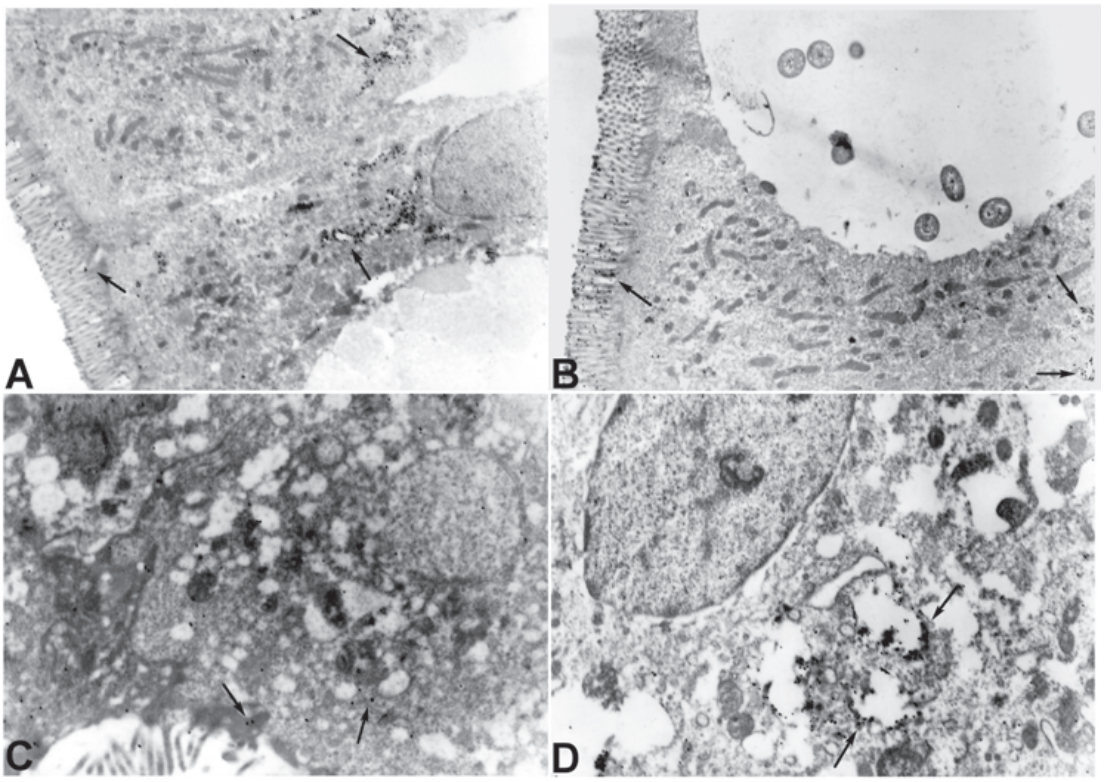


**Figure 2. Electronograms showing cyclin A expression: control (A, magnification X13000), suspected celiac disease (B, magnification X13000 ) and confirmed celiac disease (C, magnification X24000; D, magnification X27000). Arrows show immunogold-labeled cyclin A localization.**

nm and placed on nicel grids. The grids were floated on nonimmune rabbit serum (Dako) for 20 minutes and then transferred onto drops of 1:100 dilution of mouse anti human cyclin A (SIGMA). After incubation with primary antibody for 30 min, thin sections on grids were rinsed in PBS and incubated with biotinylated rabbit anti-mouse antibody (Dako) diluted 1:100. Grids were then rinsed in PBS and transferred onto drops of solution containing 10 nm gold particles conjugated to streptavidin (SIGMA) 1:20 dilution, and incubated for 30 min. After this final incubation grids were washed with PBS and dried. All incubation steps were performed at room temperature. Control specimens were incubated with nonimmune antiserum (normal mouse serum, Dako). The preparations were examined using a transmission electron microscope JEM 100 CX (JEOL, Tokyo, Japan).

## Results

*Light microscopy*

*Control group.* Biopsies taken from patients, who served as a control group, were not morphologically changed. There was cyclin A expression present in brush border and cytoplasm of epithelial cells, both in enterocytes in villi and in undifferentiated columnar cells in crypts. The strongest labeling was present near the nucleus. (fig.1a,1b)

*Suspected celiac disease.* There were no morphological changes in epithelium of investigated samples. Data obtained from light microscope showed that cyclin A was present in the brush border and cytoplasm, mostly near the nucleus. (fig.1c,1d)

*Celiac disease.* Light microscopy showed vast morphological changes. We observed either total or partial villous atrophy. Numerous lymphocytes were noticed in the lamina propria. Cross-sections of intestinal crypts showed us lack of cyclin A in the brush border. It was present in cytoplasm, however its expression was weaker in comparison with control group samples. (fig.1e,1f)

*Electron microscopy*

*Control group.* In the biopsies from controls the surface epithelium was not changed. We could observe good preservation of surface absorptive cells, which were tall, with rich columnar cytoplasm, normal-appearing mitochondria and well-shaped microvilli. Images of cells ultrastructure revealed the expression of cyclin A in brush border of enterocytes, cytoplasm – mostly near the nucleus, in form of clusters and in nucleus itself. (fig.2a)

*Suspected celiac disease.* In cases of suspected celiac disease it was shown that brush border of enterocytes remained intact. However we could observe cellular degeneration in proximity to nucleus, where cyclin A was especially expressed. Similarly to control, it was present in form of clusters. It could also be seen in the brush border and in whole cytoplasm. (fig.2b)

*Celiac disease.* Electron microscopy showed significant morphological changes in biopsies of children with celiac disease. We could often see abnormal brush border or its lack in ephitelial cells. In some cells the microvilli were much sparser, often disorientated, projected at different angles from the surface, sometimes fused or widened. There were also present abnormal lysosome-like structures and mitochondrial abnormalities. Cyclin A was rather weak expressed and it was dispersed in whole cells (fig.2c). However, in some cases, strong labeling in cytoplasm, especially near nucleus was observed (fig.2d).

Cyclin A labeling was not found in the control cells incubated with non-immune serum.

## Discussion

The aim of this study was to estimate the expression of cyclin A in the intestinal biopsy specimens from children with suspected and confirmed celiac disease to control. So far, no studies exist where this has been explored. In the present work we described our results on distribution of cyclin A in enterocytes of intestinal epithelium.

Results obtained from light and the electron microscopy showed that cyclin A was stronger expressed in biopsy specimens taken from control and suspicion groups. In these cases it was present in the brush border and in the cytoplasm, especially near the nucleus, where it formed clusters. In celiac disease samples cyclin A expression was rather weak. However, there were also some cells with strong expression of cyclin A in the cytoplasm, especially localized in the area which seem to be transformed Golgi apparatus or on structures resembling abnormal lysosomes. In other explanation these could be droplets of fat which can be the results of inability to transport resynthesized triglyceride into intracellular spaces. Cyclins are known as cell cycle proteins and the shift between nucleus and cytoplasm is necessary to regulate the passage across its different phases [25]. Cyclin A as protein synthesized and localized in the nucleus at S phase of the cell cycle plays a major role in the control of DNA replication. It has been proved that its transcription can be negatively regulated by p53 [19]. It is also known to be a marker for actively proliferating cells and for cell in S phase [26]. However, there are also data showing that cyclins may also have roles that extend beyond proliferating [18, 27–31]. In our previous studies concerning cyclin A expression in HL-60 cell line treated with doxorubicin and etoposide, we proved that cyclin A changes its function from proliferating to proapoptotic protein [18]. Furthermore, there is growing evidence that subcellular localization of cyclins may be connected with other different biological processes [17, 32–34]. Buendia et al. found that complex of cyclin- cdk2 causes an increase in the microtubule nucleating activity of centrosomes [35]. Mackay et al. showed that cyclin A inhibits intra-Golgi apparatus transport [36]. There are also data presented that cyclin B2 is involved in regulating Golgi fragmentation [37]. Noll et al. in their studies suggested that cdc25c is present in Golgi apparatus which may suggest its new role besides regulating the activity of the cyclin B/cdk1 by dephosphorylation [38]. Cyclin A is also known to be a prognostic factor in several different disease. For example Bahnassy et al. studied prognostic markers in colorectal cancer patients [39]. They found that overexpression of cyclin A and D1 correlates with poor differentiation and tumor progression. Aaltomaa et al. showed that cyclin A is a powerful and independent prognostic factor in all clinical stages of renal cell carcinoma [40]. It was also found that cyclin A can be a prognostic factor in breast cancer or in gastric carcinoma and it can be elevated in a variety of other tumors. [41–46].

Our results let us to assume that decrease of the expression and distribution of cyclin A might function as additional factor to establish terminal diagnosis for celiac disease which is particularly important because non-treated celiac disease patient were increased risk of malignancy especially lymphomas.

## References

[1] MURRAY JA. The widening spectrum of celiac disease. Am J Clin Nutr 1999; 69: 354–65.

[2] LOUGHRAN TP Jr, KADIN ME, DEEG HJ. T-cell intestinal lymphoma associated with celiac sprue. Ann Intern Med 1986; 104: 44–7.

[3] ISAACSON PG, O'CONNOR NT, SPENCER J et al. Malignant histiocytosis of the intestine:a T-cell lymphoma. Lancet 1985; 2: 688–91.

[4] HOLMES GK, STOKES PL, SORAHAN TM et al. Coeliac disease, gluten – free diet, and malignancy. Gut 1976; 17: 612–9.

[5] CELLIER C, DELABESSE E, HELMER C et al. Refractory sprue, celiac disease, and enteropathy-associated T-cell lymphoma. French Coeliac Disease Study Group. Lancet 2000; 356: 203–8.

[6] SMEDBY KE, ÅKERMAN M, HILDEBRAND H et al. Malignant lymphomas in coeliac disease: evidence of increased risks for lymphoma types other than enteropathy – type T cell lymphoma. Gut 2005; 54: 54–59.

[7] NELSEN DA. Gluten-sensitive enteropathy (celiac disease): more common than you think. Am Fam Physician 2002; 66: 2259–66.

[8] McGOWAN CH. Regulation of the eukaryotic cell cycle. Prog Cell Cycle Res 2003; 5: 1–4.

[9] Garrett MD. Cell cycle control and cancer. CurrSci 2002; 8: 515–522.

[10] WOO RA, POON RY. Cyclin-dependent kinases and S phase control in mammalian cells. Cell Cycle 2003; 2: 316–24.

[11] FROUIN I, MONTECUCCO A, BIAMONTI G et al. Cell cycle-dependent dynamic association of cyclin/Cdk complexes with human DNA replication proteins. EMBO J. 2002; 21: 2485–95.
[12] TSOU MF, STEARNS T. Controlling centrosome number: licenses and blocks. Curr Opin Cell Biol 2006; 18: 74–8.
[13] D'ASSORO AB, BUSBY R, SUINO K et al. Genotoxic stress leads to centrosome amplification in breast cancer cell lines that have an inactive G1/S cell cycle checkpoint. Oncogene 2004; 23: 4068–75.
[14] MERALDI P, LUKAS J, FRY AM et al. Centrosome duplication in mammalian somatic cells requires E2F and Cdk2-cyclin A. Nat Cell Biol. 1999; 1: 88–93.
[15] HOANG AT, COHEN KJ, BARRETT JF et al. Participation of cyclin A in Myc-induced apoptosis. Proc Natl Acad Sci USA, 1994; 91: 6875–9
[16] BORGNE A, GOLSTEYN RM. The role of cyclin-dependent kinases in apoptosis. Prog Cell Cycle Res. 2003; 5: 453–9.
[17] HIROMURA K, PIPPIN JW, BLONSKI MJ et al. The subcellular localization of cyclin dependent kinase 2 determines the fate of mesangial cells: role in apoptosis and proliferation. Oncogene 2002; 21: 1750–8.
[18] ZURYN A, GRZANKA A, STĘPIEN A et al. Expression of cyclin A in human leukemia cell line HL-60 following treatment with doxorubicin and etoposide: the potential involvement of cyclin A in apoptosis. Oncol Rep. 2007; 17: 1013–9.
[19] YAM CH, FUNG TK, POON RY. Cyclin A in cell cycle control and cancer. Cell Mol Life Sci 2002; 59: 1317–26.
[20] BORTNER DM, ROSENBERG MP. Overexpression of cyclin A in the mammary glands of transgenic mice results in the induction of nuclear abnormalities and increased apoptosis. Cell Growth Differ 1995; 6: 1579–89.
[21] BECK J, HANDGRETINGER R, DOPFER R et al. Expression of mdr1, mrp, topoisomerase II alpha/beta, and cyclin A in primary or relapsed states of acute lymphoblastic leukaemias. Br J Haematol 1995; 89: 356–63.
[22] CHAO Y, SHIH YL, CHIU JH et al. Overexpression of cyclin A but not Skp 2 correlates with the tumor relapse of human hepatocellular carcinoma. Cancer Res 1998; 58: 985–90
[23] VOLM M, KOOMÄGI R, MATTERN J et al. Stammler G. Cyclin A is associated with an unfavourable outcome in patients with non-small-cell lung carcinomas. Br J Cancer 1997; 75: 1774–8.
[24] BLEGEN H, EINHORN N, SJÖVALL K et al. Prognostic significance of cell cycle proteins and genomic instability in borderline, early and advanced stage ovarian carcinomas. Int J Gynecol Cancer 2000; 10: 477–487.
[25] JACKMAN M, KUBOTA Y, den ELZEN N et al. Cyclin A- and cyclin E-Cdk complexes shuttle between the nucleus and the cytoplasm. Mol Biol Cell 2002;13:1030–45.
[26] GELEY S, KRAMER E, GIEFFERS C et al. Anaphase-promoting complex/cyclosome -dependent proteolysis of human cyclin A starts at the beginning of mitosis and is not subject to the spindle assembly checkpoint. J Cell Biol 2001; 153: 137–48.
[27] KIYOKAWA H, NGO L, KUROSAKI T et al. Changes in p34cdc2 kinase activity and cyclin A during induced differentiation of murine erythroleukemia cells. Cell Growth Differ 1992; 3: 377–83.
[28] GAO CY, ZELENKA PS. Cyclins, cyclin-dependent kinases and differentiation. Bioessays. 1997; 19: 307–15.
[29] WELM AL, TIMCHENKO NA, ONO Y et al. C/EBPalpha is required for proteolytic cleavage of cyclin A by calpain 3 in myeloid precursor cells. J Biol Chem 2002; 277: 33848–56.
[30] HULIT J, WANG C, LI Z et al. Cyclin D1 genetic heterozygosity regulates colonic epithelial cell differentiation and tumor number in ApcMin mice. Mol Cell Biol 2004; 24: 7598–611.
[31] De FALCO M, FEDELE V, De LUCA L et al. Evaluation of cyclin D1 expression and its subcellular distribution in mouse tissues. J Anat 2004; 205: 405–12.
[32] CARBONARO-HALL D, WILLIAMS R, WU L et al. G1 expression and multistage dynamics of cyclin A in human osteosarcoma cells. Oncogene 1993; 8: 1649–59.
[33] BAILLY E, PINES J, HUNTER T et al. Cytoplasmic accumulation of cyclin B1 in human cells: association with a detergent-resistant compartment and with the centrosome. J Cell Sci 1992; 101:529–45.
[34] FAIVRE J, FRANK-VAILLANT M, POULHE R et al. Centrosome overduplication, increased ploidy and transformation in cells expressing endoplasmic reticulum-associated cyclin A2. Oncogene 2002; 21: 1493–500.
[35] BUENDIA B, DRAETTA G, KARSENTI E. Regulation of the microtubule nucleating activity of centrosomes in Xenopus egg extracts: role of cyclin A-associated protein kinase. J Cell Biol 1992; 116: 1431–42.
[36] MACKAY D, KIECKBUSCH R, ADAMCZEWSKI J et al. Cyclin A-mediated inhibition of intra-Golgi transport requires $p34^{cdc2}$. FEBS Lett 1993; 336: 549–54.
[37] JACKMAN M, FIRTH M, PINES J. Human cyclins B1 and B2 are localized to strikingly different structures: B1 to microtubules, B2 primarily to the Golgi apparatus. EMBO J. 1995; 14: 1646–54.
[38] NOLL A, RUPPENTHAL SL, MONTENARH M. The mitotic phosphatase cdc25C at the Golgi apparatus. Biochem Biophys Res Commun 2006; 351: 825–30.
[39] BAHNASSY AA, ZEKRI AR, EL-HOUSSINI S et al. Cyclin A and cyclin D1 as significant prognostic markers in colorectal cancer patients. BMC Gastroenterol 2004; 23: 4–22.
[40] AALTOMAA S, LIPPONEN P, ALA-OPAS M et al. Expression of cyclins A and D and p21(waf1/cip1) proteins in renal cell cancer and their relation to clinicopathological variables and patient survival. Br J Cancer 1999; 80: 2001–7.
[41] AHLIN C, AALTONEN K, AMINI RM et al. Ki67 and cyclin A as prognostic factors in early breast cancer. What are the optimal cut-off values? Histopathology 2007; 51: 491–8.
[42] MRENA J, WIKSTEN JP, KOKKOLA A et al. Prognostic significance of cyclin A in gastric cancer. Int J Cancer 2006; 119: 1897–901.

[43] HUSDAL A, BUKHOLM G, BUKHOLM IR. The prognostic value and overexpression of cyclin A is correlated with gene amplification of both cyclin A and cyclin E in breast cancer patient. Cell Oncol 2006; 28: 107–16.

[44] POIKONEN P, SJÖSTRÖM J, AMINI RM et al. Cyclin A as a marker for prognosis and chemotherapy response in advanced breast cancer. Br J Cancer 2005; 93: 515–9.

[45] YAM CH, FUNG TK, POON RY. Cyclin A in cell cycle control and cancer. Cell Mol Life Sci 2002; 59: 1317–26.

[46] BLEGEN H, EINHORN N, SJÖVALL K et al. Prognostic significance of cell cycle proteins and genomic instability in borderline, early and advanced stage ovarian carcinomas. Int J Gynecol Cancer 2000; 10: 477–487.